Metabolomic analysis of *Wolbachia*-infected *Aedes aegypti*

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Introduction

- Transfection of *Wolbachia* into *Aedes aegypti* successfully reduced the transmission of dengue virus in 11 countries [1]
- *Wolbachia*-infected mosquitos are released into areas at risk of mosquito transmitted disease. *Wolbachia* is maternally transmitted and attempts to form a stable infection the wild mosquito population
- **The mechanistic basis and metabolic contributions to these host-symbiont interactions are unresolved**
- We investigated the infection of two *Wolbachia* strains, *w*Mel and *w*MelPop, and two diet levels using NMR-based metabolomics (Figure 1, below)

Methods

- Two independent and distinct insect lines were used in this experiment, PGYP1 for *w*MelPop and MGYP2 for *w*Mel, each with genetically paired control lines
- At time of experiment, PGYP1 mosquitos were at 62 generations post-*w*MelPop infection and 58 generations post-tetracycline treatment
- MGYP2 mosquitoes were at 23 generations post-*w*Mel infection and 23 generations post-tetracycline treatment
- Insect lines were divided into a high and low dietary regimes
- Mosquitos were snap-frozen at 8-days post eclosion and prepared for NMR spectroscopy
- NMR samples were analysed using a 900-MHz ^{1}H-NMR spectrometer. Spectra were processed in *TopSpin* and individual signals were aligned using the *MATLAB* program *icoshift* [2]
- Spectra were data-reduced to 0.001 ppm "buckets" and normalised to total signal intensity prior to multivariate statistical analysis (MVSA) in SIMCA-P+ 15.0 (Umetrics AB, Sweden)
- Metabolite identification was conducted using Chenomx NMR Suite (v 8.3), the Human Metabolome Database, and 2D-HSQC spectra. Non-normalised NMR spectra were semi-quantified for select metabolites. Results were interpreted using the KEGG pathway map specific to *Ae. aegypti* and a *Wolbachia* genome scale model for both strains [3]

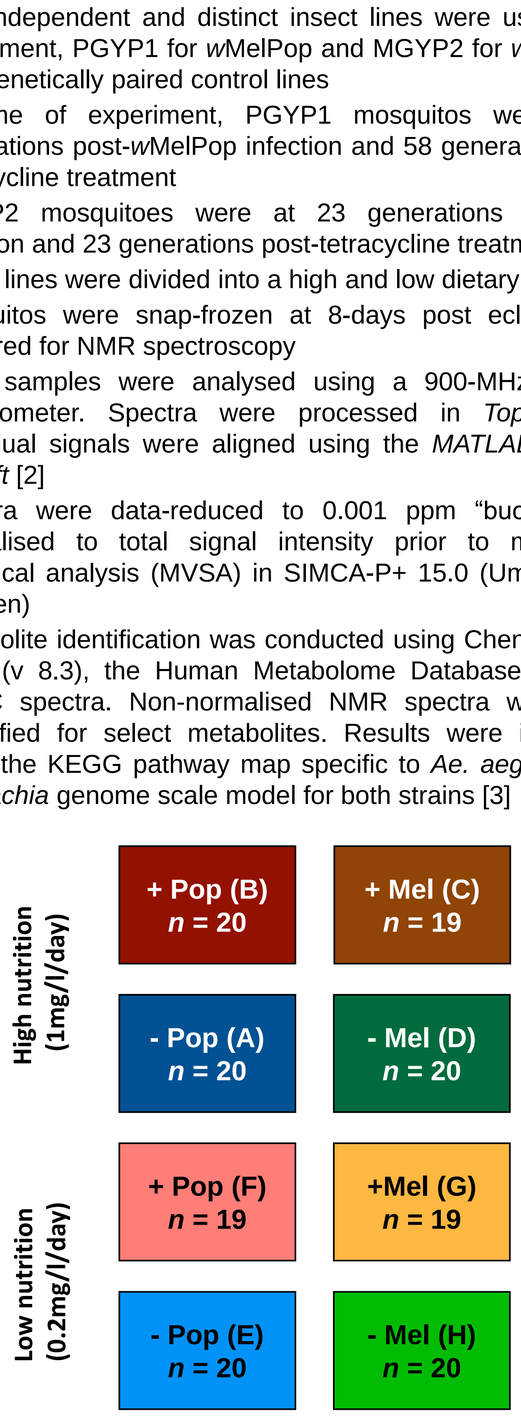

Figure 1: Schematic of experimental design. Each NMR sample was composed of five homogenised mosquitos (wings and legs removed) to provide adequate metabolic content for measurement. For supervised MVSA, groups were identified by a single categorical ***Y***-variable, A,B,C… This method does not constrain supervised models to optimise variance of experimental factors, thus preserving information about their relative strength.

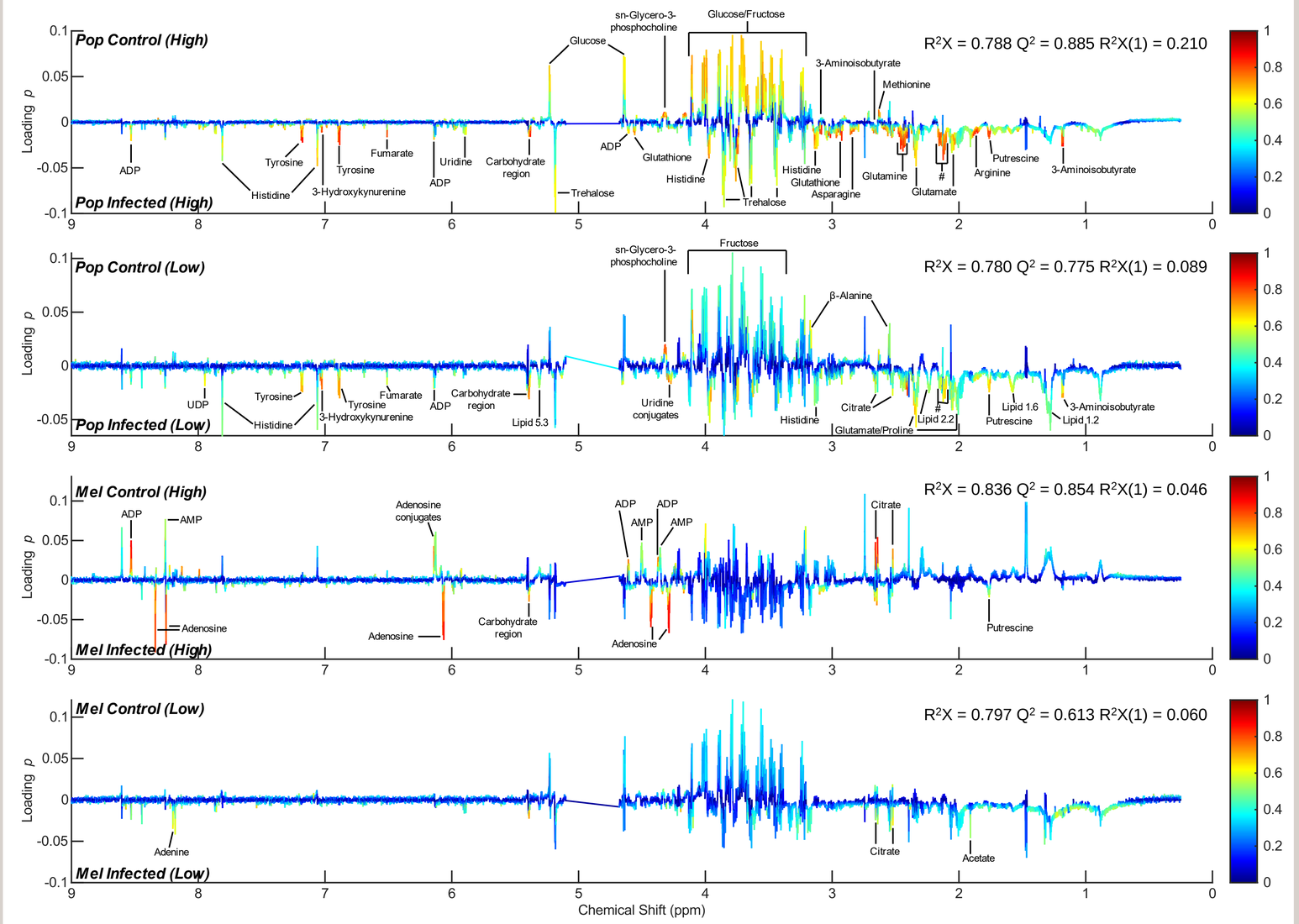

Figure 3: Heatmap S-plots of infected v. uninfected mosquitos. Spectral features representing a significant change were identified from bivariate 1D loadings plots of the first latent component for each of these key OPLS models. The loadings coefficients, *p*, were plotted against the chemical shift values and the correlation-scaled loadings coefficients, |*p(corr)*|, were superimposed on the loadings plot as a colour scaling heatmap. These plots are a 1-dimensional alternative to the S-plot that retains identical information whilst assisting metabolite identification. Variables in these plots were deemed to be significant if they had both of a loadings value |*p*| > 0.01 and a |*p(corr)*| > 0.5.

Results

- Mosquito line specific OPLS models show there are minimal overarching effects of *Wolbachia* infection, common to both strains, and diet (Figure 2, below)
  - For *w*MelPop groups, ~15% of variance in the data is attributed to infection and only ~4% is diet
  - For *w*Mel groups this is nearly reversed, ~4% of variance is attributed to infection and ~17% for diet.
- Pairwise OPLS comparisons were used to elucidate specific effects of *Wolbachia* infection, (Figure 3, above)
  - This includes increased levels of critical amino acids such as tyrosine, glutamate, glutamine, and arginine, and many other metabolites such as putrescine, histidine, fumarate, 3-aminoisobutyrate and 3-hydroxykyurenine
  - Only a few of metabolites are supressed when infected with *w*MelPop, mainly glucose
  - Comparatively, the *w*Mel infection has much less metabolic changes, mainly increased levels of adenosine or fluctuations in adenosine conjugates

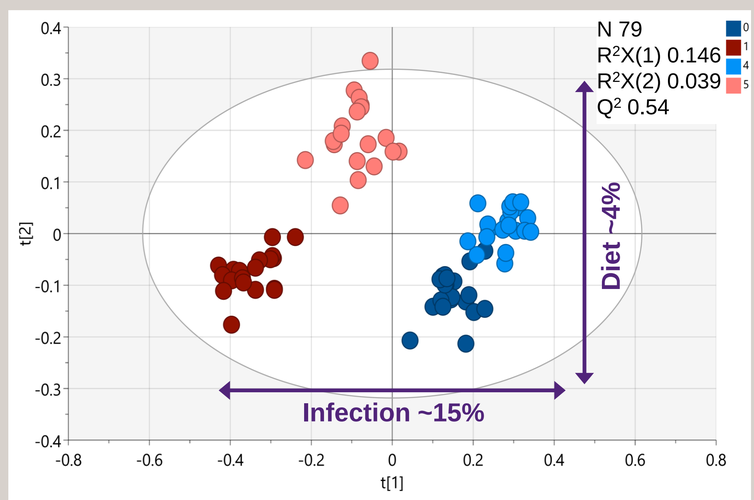

Figure 2: OPLS models of the two strains. The OPLS model fitted two components for the *w*MelPop mosquito line and shows a clear hierarchy of effects. We see a large contribution in the first component separating infected groups from uninfected, $R^2X(1)$ ~15%, and a much smaller contribution for the second component that differentiates diet regime, $R^2X(2)$ ~4%. The OPLS model for the *w*Mel mosquito line fitted three components in 'd4'-tetrahedron. The first component separates dietary regimes, $R^2X(1)$ ~17%, while the second and third components show the contributions of the high diet infected group, $R^2X(2)$ ~ 2%, and the low diet infected group, $R^2X(3)$ ~ 2%, respectively. These components effectively 'stick-out-of' and 'stick-into' the page in 3d space. T1 and T3 are shown here.

Discussion

- *Wolbachia* and its host exist in a state of perpetual tug-of-war which, given a more demanding strain of *Wolbachia*, may active the host's defence strategies [4]
- The *w*MelPop is known to be more virulent and we see clear activation of melanin immune response (MIR) pathways, via increased tyrosine in infected groups. The MIR encapsulates and destroys infected cells, evidenced by higher levels of putrescine
- We observed signs of Reactive oxygen species (ROS) production and of ROS adaption mechanisms, most likely as part of the immune response
  - We see a noticeable increase in glutathione, which is utilised in neutralising mitochondrial ROS
  - ROS can be generated in multiple ways; the MIR itself can generate ROS, *Wolbachia* competes with the host for oxygen leading to mitochondrial ROS, and the host itself may generate ROS for defence against invaders
- The lack of immune response for *w*Mel infection, and the lower metabolic impact, indicates a more stable infection that will not kill the host and thus can be inherited to offspring
- Increased adenosine in *w*Mel infected groups may indicate metabolic of provisioning of ATP to the host

References

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